

CLAIMS

1. A variant of a parent glucoamylase comprising one or more mutation(s) in the following position(s) or region(s) in the amino acid sequence shown in NO: 2:

Region: 1-18,

5 Region: 19-35,

Region: 40-62,

Region: 73-80,

Region: 93-127,

Region: 170-184,

10 Region: 200-212,

Region: 234-246,

Region: 287-319,

Region: 334-341,

Region: 353-374,

15 Region: 388-414,

Region: 445-470,

and/or in a corresponding position or region in a homologous glucoamylase which displays at least 60% homology with the amino acid sequences shown in SEQ ID NO: 2, with the exception of the following substitutions: N20C, A27C, S30P, Y48W, Y50F,

20 W52F, R54K/L, D55G/V, G57A, K108R, D112Y, Y116A/W, S119C/W/E/G/Y/P, W120H/L/F/Y, G121T/A, R122Y, P123G, Q124H, R125K, W170F, N171S, Q172N, T173G, G174C, Y175F, D176N/E, L177H/D, W178R/D, E179Q/D, E180D/Q, V181D/A/T, N182A/D/Q/Y/S, G183K, S184H, W212F, R241K, A246C, D293E/Q, A302V, R305K, Y306F, D309N/E, Y312W, W317F, E389D/Q, H391W, A392D, A393P, N395Q, G396S,
25 E400Q/C, Q401E, G407D, E408P, L410F, S411A/G/C/H/D, S460P.

2. The variant of claim 1, wherein the variant comprise one or more of the following mutations: A1V, T2E/P/Q/R/H/M, L3P/N, N9A, A11P/E, I18V, L19N, N20T, G23A, A24S/T, D25S/T/R, G26A, A27S/T, W28R/Y, S30T/N, G31A, A32V, D33R/K/H, S34N,

30 S40C, T43R, T51D/S, T53D, S56A/C, V59T/A, L60A, N93T, P94V, S95N, D97S, L98P/S, S100T/D, A102S/*, N110T, V111P, D112N, E113M/A, T114S, A115Q/G, Y116F, S119A, G127A, N182E, A201D, F202L, A203L, T204K, A205R/S, V206L/N, G207N, S208H/T/D, S209T, S211P, W212N/A/T, A246T Y312Q, N313T/S/G, A353D/S, S356P/N/D, D357S, A359S, T360V, G361S/P/T/A, T362R, S364A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/T/W/Y/V,
35 S365A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/T/W/Y/V, S366T, S368P/T/A, T369A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/T/W/Y/V, S371Y/H/N/D,

S372F/Y/C/L/P/H/R/I/T/N/S/V/A/D/G, T390R, A393R, S394R/P, M398L, S399C/Q/T, Y402F, D403S, S405T, D406N, E408C/R, L410I/R, S411V, A412C, D414A, G447S, S465P.

- 5 3. A variant of a parent glucoamylase with improved thermostability comprising one or more mutation(s) in the following position(s) or region(s) in the amino acid sequence shown in NO: 2:

Region: 1-18,

Region: 19-35,

10 Region: 73-80,

Region: 93-127,

Region: 170-184,

Region: 200-212,

Region: 234-246,

15 Region: 287-319

Region: 334-341,

Region: 353-374,

Region: 388-414.

Region: 445-470,

20 and/or in a corresponding position or region in a homologous glucoamylase which displays at least 60% homology with the amino acid sequences shown in SEQ ID NO: 2, with the exception of the following substitutions: N20C, A27C, S30P, A246C.

4. A variant of a parent glucoamylase with increased specific activity comprising one or
25 more mutation(s) in the following position(s) or region(s) in the amino acid sequence shown in NO: 2:

Region: 1-18,

Region: 40-62,

Region: 93-127,

30 Region: 170-184,

Region: 200-212,

Region: 234-246,

Region: 287-319,

Region: 388-414,

and/or in a corresponding position or region in a homologous glucoamylase which displays at least 60% homology with the amino acid sequences shown in SEQ ID NO: 2, with the exception of the following substitutions: S411G.

- 5 5. The variant according to claim 4, having one or more mutation(s) in the following region(s) in the amino acid sequence shown in NO: 2:

Region: 287-300,

Region: 305-319,

- and/or in a corresponding position or regions in a homologous glucoamylase which
10 displays at least 60% homology with the amino acid sequences shown in SEQ ID NO: 2.

6. The variant according to any of claims 1-5, wherein the parent homologous glucoamylase is the *Aspergillus niger* G1 glucoamylase.

- 15 7. The variant according to any of claims 1-6, wherein the glucoamylase is a truncated glucoamylase, in particular in the C- terminal.

8. A DNA construct comprising a DNA sequence encoding a glucoamylase variant according to any one of claims 1-7.

20 9. A recombinant expression vector which carries a DNA construct according to claim 8.

10. A cell which is transformed with a DNA construct according to claim 8 or a vector according to claim 9.

25 11. A cell according to claim 10, which is a microorganism, such as a bacterium or a fungus.

12. The cell according to claim 11, which is a protease deficient *Aspergillus oryzae* or
30 *Aspergillus niger*.

13. A process for converting starch or partially hydrolyzed starch into a syrup containing dextrose, said process including the step saccharifying starch hydrolyzate in the presence of a glucoamylase variant according to any of claims 1-7.

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14. The process of claim 14, wherein the dosage of glucoamylase is present in the range from 0.05 to 0.5 AGU per gram of dry solids.

15. The process of any claims 13 or 14, comprising saccharification of a starch hydrolyzate of at least 30 percent by weight of dry solids.

16. The process of any of the preceding claims, wherein the saccharification is conducted in the presence of a debranching enzyme selected from the group of pullulanase and isoamylase, preferably a pullulanase derived from *Bacillus acidopullulyticus* or *Bacillus deramificans* or an isoamylase derived from *Pseudomonas amyloclavata*.

17. The process of any of the preceding claims, wherein the saccharification is conducted at a pH of 3 to 5.5 and at a temperature of 60-80°C, preferably 63-75°C, for 24 to 72 hours, preferably for 36-48 hours at a pH from 4 to 4.5.

18. A method of saccharifying a liquefied starch solution, which method comprises
(i) a saccharification step during which step one or more enzymatic saccharification stages takes place, and the subsequent step of
(ii) one or more high temperature membrane separation steps
wherein the enzymatic saccharification is carried out using a glucoamylase variant according to any of claim 1 to 7.

19. Use of a glucoamylase variant according to any of claims 1-7 in a starch conversion process.

20. Use of a glucoamylase variant according to any of claims 1-7 in a continuous starch conversion process.

21. Use according to claim 20, wherein the continuous starch conversion process include a continuous saccharification process according to claim 18.

22. Use of a glucoamylase variant according to any of claims 1-7 in a process for producing oligosaccharides.

23. Use of a glucoamylase variant according to any of claims 1-7 in a process for producing specialty syrups.

24. Use of a glucoamylase variant according to any one of claims 1-7 in a process for
5 producing ethanol for fuel.

25. Use of a glucoamylase variant according to any one of claims 1-7 in a process for producing a beverage.

10 26. Use of a glucoamylase variant according to any one of claims 1-7 in a fermentation process for producing organic compounds, such as citric acid, ascorbic acid, lysine, glutamic acid.

SEQ ID NO: 2

15 27. A method for improving the thermostability and/or of increasing the specific activity of a parent glucoamylase by making a mutation in one or more of the following position(s) or region(s) in the amino acid sequence shown in NO: 2:

Region: 1-18,

Region: 19-35,

Region: 40-62,

20 Region: 73-80,

Region: 93-127,

Region: 170-184,

Region: 200-212,

Region: 234-246,

25 Region: 287-319,

Region: 334-341,

Region: 353-374,

Region: 388-414,

Region: 445-470,

30 and/or in a corresponding position or region in a homologous glucoamylase which displays at least 60% homology with the amino acid sequences shown in SEQ ID NO: 2.

28. The method according to claim 27, having one or more of the following substitutions:
A1V, T2E/P/Q/R/H/M, L3P/N, N9A, A11P/E, I18V, L19N, N20T, G23A, A24S/T,
35 D25S/T/R, G26A, A27S/T, W28R/Y, S30T/N, G31A, A32V, D33R/K/H, S34N, S40C,
T43R, T51D/S, T53D, S56A/C, V59T/A, L60A, N93T, P94V, S95N, D97S, L98P/S,

S100T/D, A102S/*, N110T, V111P, D112N, E113M/A, T114S, A115Q/G, Y116F, S119A, G127A, N182E, A201D, F202L, A203L, T204K, A205R/S, V206L/N, G207N, S208H/T/D, S209T, S211P, W212N/A/T, A246T Y312Q, N313T/S/G, A353D/S, S356P/N/D, D357S, A359S, T360V, G361S/P/T/A, T362R, S364A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/T/W/Y/V,
5 S365A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/T/W/Y/V, S366T, S368P/T/A, T369A/R/N/D/C/Q/E/G/H/I/L/K/M/F/P/T/W/Y/V, S371Y/H/N/D, S372F/Y/C/L/P/H/R/I/T/N/S/V/A/D/G, T390R, A393R, S394R/P, M398L, S399C/Q/T, Y402F, D403S, S405T, D406N, E408C/R, L410I/R, S411V, A412C, D414A, G447S, S465P.

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